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Age specific trends in asthma mortality in England and Wales, 1983-95: results of an observational study

M J Campbell, G R Cogman, S T Holgate, S L Johnston

Abstract

Objective: To determine trends in asthma mortality by age group in England and Wales during 1983-95.

Design: Observational study.

Setting: England and Wales.

Subjects: All deaths classified as having an underlying cause of asthma registered from 1 January 1983 to 31 December 1995.

Main outcome measure: Time trends for age specific asthma deaths.

Results: Deaths in the age group 5-14 years showed an irregular downward trend during 1983-95; deaths in the age groups 15-44, 45-64, and 65-74 years peaked before 1989 and then showed a downward trend; and deaths in the age group 75-84 years peaked between 1988 and 1993 and subsequently dropped. Trends were: age group 5-14 years, 6% (95% confidence interval 3% to 9%); 15-44 years, 6% (5% to 7%); 45-64 years, 5% (4% to 6%); 65-74 years, 2% (1% to 3%). Deaths in the 75-84 and 85 and over categories plateaued.

Conclusions: There are downward trends in asthma mortality in Britain, which may be due to increased use of prophylactic treatment.

Introduction

It is now over 10 years since trends and seasonality in asthma mortality were investigated in England and Wales,¹⁻³ and the long term trends over the period 1974-84 were found to be increasing. We aimed at updating these findings in the light of changing views on the appropriateness of treatment for asthma, increasing concerns in relation to environmental effects on asthma, and also concerns on admission rates for asthma.⁴

Method

The data were obtained from the Office of Population Censuses and Surveys (now the Office for National Statistics). They consisted of all deaths classified as having an underlying cause of asthma (International Classification of Diseases, ninth revision (ICD-9), codes 493.0 to 493.9 inclusive) registered in England and Wales from 1 January 1983 to 31 December 1995. Some coding procedures were changed in 1983, so the data were analysed including and excluding that year

to see if the change affected conclusions. Data included the date of death, cause of death, sex of subject, and age at death. Annual age specific population sizes and death rates for all respiratory deaths (ICD-9 codes 460-519) were obtained from Office of Population Censuses and Surveys publications.

For analysis the data were split into seven age groups, coinciding with the Office of Population Censuses and Surveys age classifications 0-4, 5-14, 15-44, 45-64, 65-74, 75-84, and 85 years and over. The number of people alive during each year in these age groups was obtained from census projections. Deaths were aggregated into years. The appendix describes the method of analysis.

Results

In total, 23 311 asthma deaths were registered between 1 January 1983 and 31 December 1995. The proportions of these deaths in each age group 0-4, 5-14, 15-44, 45-64, 65-74, 75-84, and 85 and over were 0.5%, 1%, 12%, 27%, 26%, 24%, and 10% respectively.

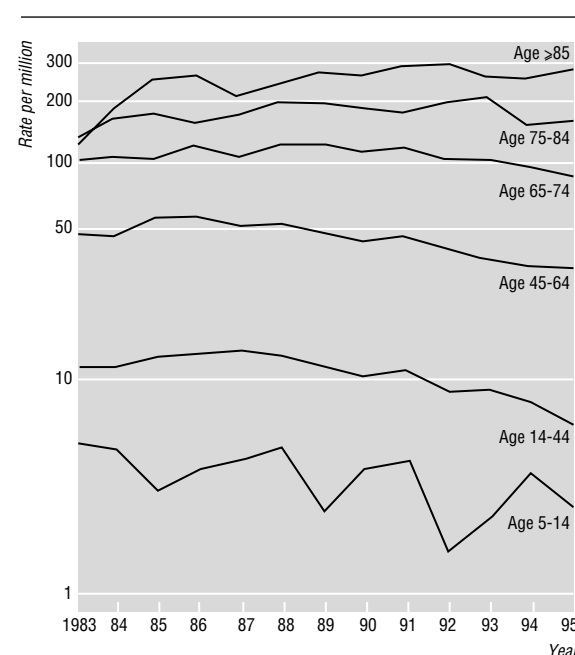


Fig 1 Death rates from asthma per million population by age group from 1983 to 1995

See editorial by Woolcock

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Table 1 Trends in asthma mortality 1983-95

Age group (years)	No of deaths	Linear and quadratic coefficients	Estimate†	SE	P value
5-14	277	Time	-0.0593	0.0164	0.001
		Time ²	0.0005	0.0049	0.914
15-44	2874	Time	-0.0598	0.0054	<0.001
		Time ²	-0.0091	0.0016	<0.001
45-64	6185	Time	-0.0489	0.0036	<0.001
		Time ²	-0.0071	0.0011	<0.001
65-74	6005	Time	-0.0190	0.0036	<0.001
		Time ²	-0.0062	0.0011	<0.001
75-84	5717	Time	0.0038	0.0037	0.31
		Time ²	-0.0065	0.0011	<0.001
≥85	2253	Time	0.0311	0.0062	<0.001
		Time ²	-0.0076	0.0018	<0.001

†Coefficient from Poisson regression for annual asthma deaths.

Figure 1 shows the yearly asthma death rates plotted on a log scale for each age group. Table 1 gives the results of the Poisson analysis. Little could be made of the data for the under 5s because the numbers were too small. For all age groups except 5-14 there was a significant quadratic term. For each age group between 15 and 74 both linear and quadratic terms were negative, implying an accelerating decline which started before 1989. For subjects aged 75-84 the linear term was positive, suggesting that mortality initially rose and then either peaked or plateaued after 1989. Visual inspection of the data suggests that for those aged 75-84 there was a drop in 1994 and 1995, but for those aged 85 and over no decline was evident.

Excluding 1983 made little difference to the results. As the model was on a log scale, disregarding the quadratic term we can interpret the linear coefficients as a proportionate drop. Hence for deaths between 5 and 14 years of age the drop was about 6% a year (95% confidence interval 3% to 9%), for deaths at ages 15-44 years it was also 6% (5% to 7%), for deaths at ages 45-64 years it was 5% (4% to 6%), and for deaths at ages 65-74 years it was 2% (1% to 3%). For deaths in subjects aged 75 and over the rate was flat.

Total respiratory deaths also decreased after about 1991 in subjects aged 5-14 and 15-44. In 1991 as a proportion of all respiratory deaths asthma deaths accounted for 44% among subjects aged 5-14 years, 31% among those aged 15-44, 11% among those aged 45-64, and 2% among those over 65.

Discussion

In contrast with studies up to the mid-1980s¹⁻³ which showed increasing mortality, we have shown that since the late 1980s asthma mortality in England and Wales seems to have dropped except among people aged over 85. Other countries have had different experiences. In Scotland mortality was stable between 1975 and 1989 for 5-44 year olds.⁵ In France a peak in mortality for both the under 35s and over 35s was observed from 1985 to 1987; this was attributed to influenza epidemics, and though death rates were lower subsequently there was no evidence of a trend.⁶

Key messages

- Asthma mortality in England and Wales is dropping by about 6% a year in people aged 5-64 years
- It is changing only slowly in those aged 65 and over

In contrast, deaths in New Zealand at ages 5-54 years showed a downward trend from 1986 to 1992.⁷

Trends in asthma mortality among children are reflected in the trends in asthma admissions to hospital. Routine data for asthma admissions are not available for England after 1985, but for Wales admissions showed a steady rise from 1983 to 1988 and then a drop in 1989 and 1990.⁴ Age standardised death rates seem to reflect this trend.

There has been considerable concern in the medical community and in the public domain over reports of increasing asthma prevalence.⁸ It is encouraging to note that even with a background of increasing prevalence there were downward trends in mortality in the under 75s in the five to seven years before 1995. It is possible that these trends were a result of increased awareness among physicians and patients of the inflammatory basis of asthma and the need for prophylactic treatment, particularly in view of the increased prescribing of corticosteroids as a proportion of all prescriptions for asthma.⁹ Diagnostic transfer is a possibility, but it is reassuring that respiratory deaths are also dropping in younger people. The accuracy of death certification in asthma was not good in 1979 but has been shown to be more accurate for younger people.¹⁰ In old people asthma deaths form only a small proportion of all respiratory deaths and it would be impossible to quantify the extent of diagnostic transfer.

The trends in asthma mortality may be related to the increased use of prophylactic treatment, the use of which should continue to be encouraged.

We thank the Office for National Statistics (formerly the Office of Population Censuses and Surveys) for the data and Mr S A Julious for additional references.

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Conflict of interest: None.

Appendix

Analysis was by Poisson regression in STATA.¹¹ The midyear population was included as an "offset," which ensured the analysis was based on a rate and allowed for changes in the age structure of the population. Checks for overdispersion and serial correlation of the residuals were carried out but it was not found necessary to make allowances for them. Trend terms were centred on the midyear 1989. When the linear and quadratic terms were negative this implied that the fitted model peaked before 1989. When the linear term was positive and the quadratic term was negative this implied that the model had peaked or plateaued or was expected to peak or plateau after 1989. When both coefficients were positive an increasing and accelerating rate was implied.

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Effect of long term treatment with salmeterol on asthma control: a double blind, randomised crossover study

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Abstract

Objectives: To determine the effect of adding salmeterol 50 µg twice daily for six months to current treatment in subjects with asthma who control their inhaled corticosteroid dose according to a management plan.

Design: A double blind, randomised crossover study.

Setting: Nottingham.

Subjects: 101 subjects with mild or moderate asthma taking at least 200 µg twice daily of beclomethasone dipropionate or budesonide.

Interventions: Salmeterol 50 µg twice daily and placebo for six months each, with a one month washout. Subjects adjusted inhaled steroid dose according to guidelines.

Main outcome measure: Reduction in inhaled steroid use, exacerbations of asthma, and use of oral steroids.

Results: Data were available for 87 subjects. When compared with placebo salmeterol treatment was associated with a 17% reduction in inhaled steroid use (95% confidence interval 12% to 22%) with no significant difference in the number of subjects who had an exacerbation (placebo 25%, salmeterol 16%) or use of oral steroids. For secondary end points salmeterol treatment was associated with higher morning and evening peak expiratory flow and forced expiratory volume in one second; a reduction in symptoms, bronchodilator use, and airway responsiveness to methacholine; and no effect on serum potassium concentration, 24 hour heart rate, or the final forced expiratory volume in one second achieved during a salbutamol dose-response study.

Conclusions: In subjects who adjusted their inhaled steroid treatment according to guidelines the addition of salmeterol 50 µg twice daily was associated with a reduction in inhaled steroid use and improved lung function and symptom control.

Introduction

Salmeterol, a long acting β_2 agonist, when inhaled twice daily, causes bronchodilatation that is maintained over 24 hours.^{1 2} The findings by Sears *et al* that asthma control was worse when subjects took the short acting

β_2 agonist fenoterol regularly rather than a β_2 agonist as required³ led to concerns that regular treatment with long acting β_2 agonists might have similar adverse effects. Subsequent studies comparing salmeterol 50 µg twice daily with placebo have shown that salmeterol causes bronchodilatation that is maintained for at least three months^{4 5} and an improvement in quality of life⁶ and symptom control.⁴⁻⁹ When compared with placebo or salbutamol, however, salmeterol has usually not reduced exacerbations,^{4 5 8-11} nor does it reduce inflammation in asthmatic airways.^{12 13}

Current recommendations suggest that treatment for asthma should be modified according to symptoms and peak expiratory flow measurements.^{14 15} The introduction of a long acting β_2 agonist may therefore lead to a reduction in inhaled corticosteroid use. Previous studies have looked at the effect of adding salmeterol when inhaled steroid dose is kept constant. We determined the effect of adding salmeterol to other treatment on the basis of current practice, with subjects changing their inhaled steroid dose according to predetermined criteria based on symptoms and peak expiratory flow. We studied the long term efficacy and safety of salmeterol in subjects with mild or moderate asthma who took salmeterol and placebo for six months each.

Methods

Subjects

We recruited 101 subjects (50 female) aged 19-60 years from our register of asthma volunteers and from outpatient clinics in Nottingham and Mansfield. The subjects had to have a forced expiratory volume in one second of at least 50% predicted and either (a) a 15% increase in forced expiratory volume in one second after inhaling salbutamol 400 µg at entry or within a year or (b) 15% diurnal variability in peak expiratory flow recordings. All were receiving an inhaled short acting β_2 agonist as needed and at least 400 µg/day beclomethasone dipropionate or budesonide. Two subjects were taking ipratropium bromide and two theophylline, both in constant dose throughout the study. All had stable asthma at entry, with no exacerbations or respiratory tract infection in the previous six

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weeks. Subjects gave written informed consent; the study was approved by Nottingham City Hospital's ethics committee.

Measurements

We measured forced expiratory volume in one second and forced vital capacity with a dry bellows spirometer, the Vitalograph (Vitalograph, Buckingham), as the best of three readings within 100 ml for forced expiratory volume in one second. We measured peak expiratory flow with a mini-Wright peak flow meter (Clement Clarke, Harlow) as the best of three readings. A salbutamol dose-response curve was obtained by the patient inhaling salbutamol at 15 minute intervals to provide cumulative doses of 100 µg, 200 µg, 400 µg, and 800 µg. We measured bronchial reactivity using a modified method of Yan *et al.*¹⁶ with the subjects inhaling three puffs of saline followed by doubling doses of methacholine from 0.048 µmol to 196 µmol. We measured forced expiratory volume in one second one minute after each dose, and the test was stopped when it had fallen by at least 20%. The provocative dose of methacholine causing a 20% fall in forced expiratory volume in one second (PD₂₀) was calculated by linear interpolation of the last two readings on the log dose-response plot. The subjects took no study drugs within eight hours, no short acting β₂ agonists within four hours, and no ipratropium bromide within 24 hours of all visits.

The subjects recorded peak expiratory flow before taking the study drug, rescue bronchodilator use, and day and night-time symptom scores (0 = no symptoms, 4 or 5 = severe symptoms) twice daily throughout the study.

Protocol

This was a double blind, crossover study in which the subjects inhaled placebo and salmeterol 50 µg twice daily from identical dry powder inhalers (Diskhaler with Rotadisk; GlaxoWellcome, Greenford) for 24 weeks followed by a four week washout, the order of treatment being randomised by computer program. We assessed asthma control after a two week run-in when the subjects recorded peak expiratory flow and symptom scores. If control was considered reasonable by both the doctor and the subject an individualised "target" peak expiratory flow representing good control of asthma was determined and the subject issued with a personalised management plan (see below). Otherwise the steroid dose was increased or reduced by one puff twice daily with further review after four weeks, or for longer if necessary, until their asthma was stable. One puff could be 100 µg, 200 µg, 250 µg, or 400 µg of beclomethasone dipropionate or budesonide according to the inhaler used (which was constant throughout the study). The subjects requiring less than 200 µg beclomethasone dipropionate or budesonide twice daily were excluded from entry.

We measured forced expiratory volume in one second and forced vital capacity every four weeks during the 56 week study and determined the number of used and unused Rotadisks to assess compliance. We measured PD₂₀ methacholine and serum potassium concentration after 12 and 24 weeks of each treatment, carried out a salbutamol dose-response study at 20 weeks (both at least 8 hours after study treatment), and

performed 24 hour electrocardiography by Holter monitor during weeks 1 and 24. A 24 hour contact telephone number and physician with a radiopager were available throughout the study.

Asthma management plan

We adjusted treatment at clinic visits and between visits with written and oral instruction, which was reinforced at each visit. When the subjects had had no symptoms and a morning peak expiratory flow greater than or equal to their target peak expiratory flow for 14 consecutive days they reduced their inhaled steroid dose by one puff twice daily; they were not allowed, however, to reduce the dose by more than one puff twice daily below the dose at entry. If morning or evening peak expiratory flow fell by 10% on two consecutive days or by 15% on one day subjects increased their inhaled steroid by two puffs twice daily for at least one week, adding 30 mg prednisolone daily if peak expiratory flow fell by 30%. The additional treatment was continued until symptom scores and peak expiratory flow had returned to previous levels, when it was reduced by one puff twice daily per week to the previous dose or by 5 mg prednisolone every two days; if a subject's condition deteriorated the higher dose was maintained.

Exacerbations were defined by the presence of any two of the following: a 30% fall in morning peak expiratory flow from target peak expiratory flow; a fall in forced expiratory volume in one second of either 0.7 litres or 30% from baseline; increased use of β₂ agonist (by more than four puffs a day); the need for oral prednisolone; more treatment on two consecutive nights or increased symptom scores on two successive days.

Analysis

Primary end points were mean daily inhaled steroid dose, number of exacerbations, and courses of oral prednisolone over the last five months of treatment. Parametric analyses were performed on mean values of forced expiratory volume in one second; morning and evening peak expiratory flow; minimum, mean, and maximum heart rate; and log transformed values for daily inhaled steroid dose, serum potassium concentrations, and PD₂₀ methacholine. PD₂₀ values were included only if measured within 8-14 hours of the study drug (66% of subjects). Reversibility to salbutamol was characterised by mean initial and maximum forced expiratory volume in one second and mean increase in forced expiratory volume in one second. For end points measured over the last five months of treatment, all the subjects with data for at least one day of treatment were included in the analyses. Initially, an analysis of variance model was fitted containing terms for the main effects of baseline (mean over last two weeks of the run-in when appropriate), treatment, period, carry over, subject within sequence (order of drug randomisation), and a baseline by treatment interaction.¹⁷ The effects of baseline, carry over, and interactions were removed if non-significant at the 10% level; the main model then fitted included terms for subject, period, and treatment only.

All remaining outcomes including the percentage of days (24 hours) without use of a bronchodilator and symptom free days and nights were analysed non-parametrically by Wilcoxon's rank sum test to

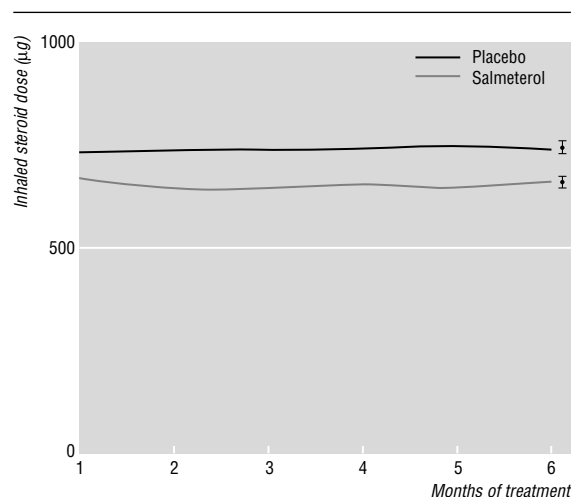


Fig 1 Geometric mean inhaled steroid dose during treatment with salmeterol and placebo, with standard error bars

establish the significance of treatment and carry over effects. Differences in numbers of exacerbations and courses of oral steroids were compared with Prescott's test.¹⁷

Results

Of the 101 subjects who entered the study, 14 subjects failed to complete the study owing to protocol violations (three), pregnancy (three), personal reasons (five), and adverse events (three; see results). Data for the efficacy analysis were available for 87 subjects, including one subject who moved house during month 10. Mean compliance was 92%, with 67% and 64% of subjects achieving more than 90% compliance with placebo and salmeterol respectively.

Subjects consisted of 51 men and 50 women with a mean age of 39 (SD 10) years; 50 had never smoked, 39 were former smokers, and 12 were current smokers. Most of the 101 subjects had a positive skin test to house dust mite (71%), cat (79%), or grass pollen (56%). Table 1 shows the subjects' lung function.

Baseline measurements of subjects were similar regardless of which drug they had been randomised to receive first—salmeterol ($n=51$) or placebo ($n=50$) (table 1). There were no carry over effects (all $P>0.15$) apart from minimum heart rate ($P=0.09$), which was excluded from further analysis. The only significant interaction between treatment and baseline measurements was a weak interaction with baseline inhaled steroid dose ($P=0.10$).

Primary end points

Geometric mean inhaled steroid use was stable over the last five months of each treatment (fig 1) and significantly lower with salmeterol (561 µg) than with placebo (674 µg); the average difference was 17% (95% confidence interval 12% to 22%, $P<0.001$). Analysis according to baseline inhaled steroid dose showed that the 45 subjects initially taking 600 µg or less had a 19% lower inhaled steroid dose with salmeterol (329 µg) than with placebo (404 µg) (12% to 25%), whereas the 42 subjects taking more than 600 µg inhaled steroid initially showed a 14% difference (6% to 21%; 998 µg v 1162 µg inhaled steroid).

Table 1 Baseline characteristics of subjects receiving salmeterol first (sequence 1) and placebo first (sequence 2) and of all subjects combined

	Sequence 1 ($n=51$)	Sequence 2 ($n=49^*$)	All subjects ($n=100^\dagger$)
Mean (SD) forced expiratory volume in one second (l)	2.74 (0.82)	2.69 (0.75)	2.71 (0.79)[80‡]
Mean (SD) peak expiratory flow (l/min):			
Morning	415 (97)	415 (106)	415 (101)[80‡]
Evening	439 (95)	423 (102)	431 (98)[83‡]
Geometric mean (range) daily dose inhaled steroid (µg)	701 (400-2000)	721 (400-3000)	711 (400-3000)
Median (range) symptom score:			
Day	0.5 (0-3)	0 (0-3)	0 (0-3)
Night	0 (0-2)	0 (0-2.5)	0 (0-2.5)
Median % (range) of symptom free days	50 (0-100)	62 (0-100)	56 (0-100)
Median % (range) of symptom free nights	85 (0-100)	100 (0-100)	93 (0-100)
Median (range) bronchodilator use (puffs/day)	3 (0-11)	2 (0-14)	2.5 (0-14)
Median % (range) of bronchodilator free days	7 (0-100)	14 (0-100)	8 (0-100)

* $n=50$ for forced expiratory volume in one second.

† $n=101$ for forced expiratory volume in one second.

‡Percentage predicted.

Most subjects had no exacerbation ($n=52$) and needed no oral steroids ($n=66$) during the study, and most of the remaining subjects had one exacerbation or one course of oral steroids only (table 2). Oral steroids were used more often in the first six months than the second six months with both placebo (8 v 5 subjects) and salmeterol (7 v 2).

With salmeterol and placebo 16 and 25 subjects respectively had an exacerbation, and 9 and 13 subjects had a course of oral steroids; neither difference was significant. Most (27/34) of the episodes in which subjects took oral steroids fulfilled the criteria for an exacerbation; some exacerbations were not treated with oral steroids.

Secondary end points

Lung function and bronchial responsiveness

Table 3 and figure 2 show that salmeterol was associated with higher peak expiratory flow values compared with placebo in both the morning and evening and that forced expiratory volume in one second and forced vital capacity were also significantly higher with salmeterol.

Geometric mean values for PD₂₀ methacholine were significantly higher after salmeterol than placebo (table 3). The salbutamol dose-response study showed a higher initial forced expiratory volume in one second (2.82 v 2.71 litres, $P<0.001$) and a smaller increase in

Table 2 Oral steroid use and asthma exacerbations during last five months of treatment with placebo and salmeterol

	No of subjects	
	Placebo	Salmeterol
No of discrete courses of steroids:		
None	74	78
1	7	7
2	5	1
3	0	0
4	1	1
No of asthma exacerbations:		
None	62	71
1	20	13
2	2	2
3	1	1
>3	2	0

Table 3 Mean values for secondary end points after placebo and salmeterol

	Salmeterol	Placebo	Difference (95% CI)	Significance
Forced expiratory volume in one second (l)	2.84	2.71	0.13 (0.085 to 0.174)	P<0.001
Forced vital capacity (l)	4.01	3.89	0.12 (0.058 to 0.179)	P<0.001
Peak expiratory flow (l/min):				
Morning	451	431	19 (14 to 24)	P<0.001
Evening	456	440	16 (11 to 22)	P<0.001
PD₂₀ methacholine* (μmol):				
3 Months	3.20	2.26	0.5 (0.03 to 0.99)†	P=0.04
6 Months	2.24	1.46	0.62 (0.18 to 1.05)†	P=0.008
Heart rate (beats/min):				
Mean:				
Week 1	77	75	1 (0 to 3)	P=0.1
Week 24	78	77	1 (-1 to 3)	P=0.3
Maximum:				
Week 1	131	135	-3 (-8 to 1)	P=0.1
Week 24	133	129	4 (-1 to 8)	P=0.1
Serum potassium (mmol/l):				
3 Months	4.00	3.94	1.02 (0.99 to 1.04)‡	P=0.1
6 Months	3.99	3.92	1.02 (0.99 to 1.05)‡	P=0.3

Mean values for forced expiratory volume in one second and forced vital capacity are for the last five months of treatment; those for PD₂₀ methacholine and serum potassium concentration are geometric means.

*Provocative dose causing a 20% fall in forced expiratory volume in one second.

†Difference in doubling doses.

‡Antilogged differences in serum potassium concentration are expressed as a ratio.

forced expiratory volume in one second (11% *v* 16%) during the salmeterol treatment period compared with placebo, but the maximum forced expiratory volume in one second achieved was almost identical (3.11 *v* 3.10 litres, *P* = 0.68) (fig 3).

Symptom scores and use of bronchodilator

The subjects had more symptom free days and nights and used less relief bronchodilation with salmeterol. The median percentages for salmeterol and placebo were 96% and 86% for symptom free nights, 82% and 64% for symptom free days, and 73% and 58% for

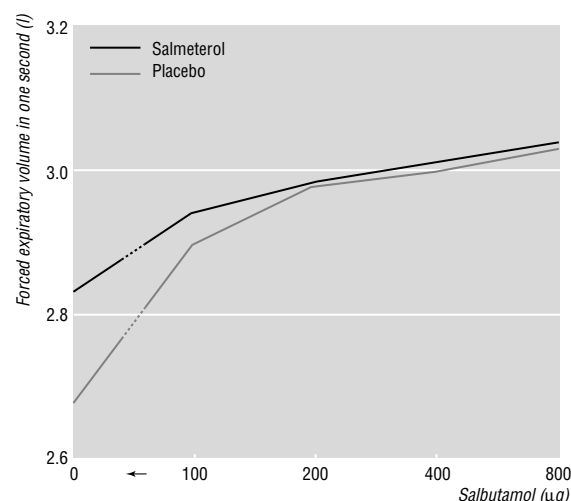


Fig 3 Mean forced expiratory volume in one second in response to increasing doses of salbutamol following salmeterol and placebo. The dose-response studies were carried out at least eight hours after the last dose of salmeterol

bronchodilator free days (all differences significant at *P* < 0.001).

Safety aspects

Serum potassium concentrations with salmeterol and placebo did not differ significantly (table 3). The quality of the 24 hour electrocardiography on all four occasions was sufficient to be able to assess maximum heart rate in 60 subjects and mean heart rate in 47. There were no significant differences in mean or maximum 24 hour heart rate between placebo and salmeterol (table 3) and no difference in the frequency distribution of ventricular and supraventricular ectopic beats.

Three subjects withdrew from the study because of adverse events, two during or after salmeterol treatment (chest pain and hair loss), and one during placebo (headache).

Salmeterol was associated with more palpitations (4 *v* 1); other expected adverse effects occurred infrequently with placebo (cramp 2, hypokalaemia 1, tremor 1) but not with salmeterol treatment.

Discussion

Long term regular treatment with the short acting β_2 agonists has provided no perceptible clinical benefit in most studies.^{3-6 18 19} Concern that they may have deleterious effects emphasised the need for long term studies of the long acting β_2 agonists.³ Recent studies suggest that when added to other treatment the long acting β_2 agonists improve asthma control compared with placebo⁴⁻¹¹ and salbutamol.⁴⁻⁶ Our study design differed from previous studies in that the subjects were asked to adjust their inhaled steroid intake within certain limits according to their peak flow rate and symptoms. This design was chosen to ensure that subjects were not undertreated or overtreated with steroids for a long period and to mimic what is likely to happen in clinical practice when salmeterol is introduced.^{14 15} The subjects were taking different types and different strengths of steroid inhaler, but these remained constant for an individual throughout the study.

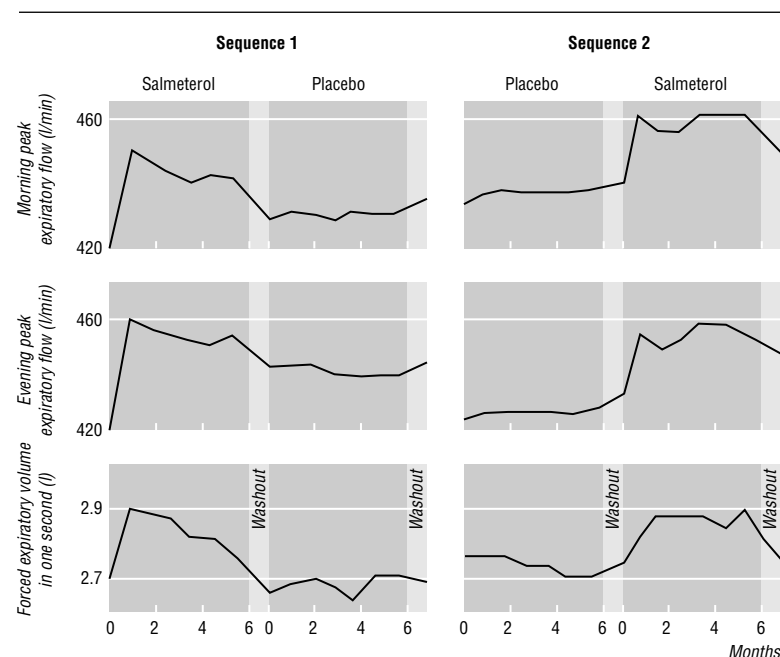


Fig 2 Mean morning and evening peak flow and forced expiratory volume in one second during treatment with salmeterol and placebo, according to whether salmeterol was given first (sequence 1) or second (sequence 2)

The subjects followed their management plan well, and the relatively low incidence of exacerbations suggests that it was effective.

By basing the study on a single centre we aimed to ensure high compliance with treatment and a low withdrawal rate as this can be an important source of bias. Data were available for 87 of the 101 subjects, and mean compliance with treatment was 92%. Subjects who failed to complete did so largely for personal reasons, with no difference between salmeterol and placebo in the number or reasons for withdrawal.

The addition of salmeterol for six months was associated with a reduction in inhaled steroid use and symptoms and an increase in forced expiratory volume in one second and morning and evening peak expiratory flow. We have presented the findings over the last five months of treatment to reduce any crossover effects and to allow time for subjects to adjust their inhaled steroid dose in response to change in treatment. We found no carry over effects apart from minimum heart rate, which was omitted from further analysis, and no treatment baseline interactions except for a weak relation with baseline log inhaled steroid dose, which we explored further.

The mean inhaled steroid dose was 17% lower with salmeterol than with placebo during the last five months of treatment. The subjects on lower doses of inhaled steroid initially had a slightly higher percentage fall in inhaled steroid dose with salmeterol, though there was considerable overlap with those initially on a higher dose. The 17% reduction should not be interpreted as the steroid sparing dose of salmeterol as subjects had limited freedom to reduce their inhaled steroid dose (by two puffs a day maximum from the dose at randomisation so that the minimum dose possible was 100 µg twice daily). If all subjects had reduced their dose to the minimum allowed the geometric mean dose would have been 387 µg (rather than 561 µg). The decision to limit the reduction in dose was based on current consensus guidelines^{14 15} and an appreciation that peak expiratory flow is only one measure of asthma control. Salmeterol was associated with fewer symptoms and no difference in exacerbations or use of rescue oral steroids compared with placebo, despite the reduction in inhaled steroid dose.

Relatively few subjects had an exacerbation during the study, probably because the management plan ensured early detection of deterioration and encouraged prompt treatment. Most studies comparing salmeterol with placebo or a short acting β_2 agonist have found no difference in exacerbations, although most had insufficient power to detect this.^{4 5 8-11} A large surveillance study found a smaller reduction in withdrawals owing to asthma with salmeterol compared with salbutamol, although the trend for deaths was in the opposite direction.²⁰ Airway inflammation is not reduced by salmeterol or the shorter acting β_2 agonists compared with inhaled steroids^{12 13}; whether this is relevant to the frequency or severity of exacerbations is uncertain.

This is the first controlled study to compare salmeterol 50 µg twice daily with placebo for more than three months. The effect of salmeterol was maintained over six months, with no evidence of tolerance for any clinical end point. When we looked at bronchodilator responsiveness to salbutamol baseline

Key messages

- One hundred and one subjects with mild or moderate asthma took salmeterol 50 µg and placebo for six months each in a crossover study
- Subjects adjusted their inhaled steroid dose according to a management plan based on peak flow recordings and symptoms
- The dose of inhaled steroids was reduced by 17% with salmeterol, with no change in exacerbations or use of oral steroids
- Despite the reduction in inhaled steroid dose, salmeterol was associated with bronchodilatation and a reduction in symptoms
- The efficacy of salmeterol was maintained over the six months

forced expiratory volume in one second was higher with salmeterol, but the final forced expiratory volume in one second achieved was identical in the two treatment periods, as in previous studies.^{21 22} We interpret this as showing that bronchodilator responsiveness is maintained after regular salmeterol treatment.

The PD₂₀ methacholine was higher after salmeterol than placebo, although the differences were small. The magnitude of the effect will be affected by the timing of the measurement, 8-14 hours after treatment, when some residual effect of salmeterol would be expected, and possibly by the development of tolerance, as seen with other β_2 agonists^{23 24} as well as salmeterol.^{25 26}

Salmeterol, like other β_2 agonists, causes systemic effects such as hypokalaemia and tremor when given in high doses.^{27 28} Such effects are rare with a 50 µg twice daily dose,^{8 28} and there was no difference in adverse effects, serum potassium concentrations, or 24 hour heart rate between treatments.

The design of this study is relevant to how salmeterol is likely to be used in clinical practice, and the findings provide some reassurance that if salmeterol is introduced at an earlier point in treatment¹⁴ (at step 3 rather than step 4 of the guidelines for treatment of asthma) a subsequent reduction in inhaled steroid dose is unlikely to have a detrimental effect in patients who followed an appropriate management plan.

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Conflict of interest: None. (The study was designed and analysed by the authors, and the results were checked against a similar analysis carried out by GlaxoWellcome.)

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Molecular investigation into outbreak of HIV in a Scottish prison

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Abstract

Objectives: To support already established epidemiological links between inmates of Glenochil prison positive for HIV infection by using molecular techniques and thus provide evidence of the extent of acquisition during a recent outbreak of the disease resulting from needle sharing. To identify possible sources of the outbreak, and to demonstrate the ability of the methodology to make further links beyond the original outbreak.

Design: Viral sequences obtained from the blood of HIV positive prisoners previously identified by standard epidemiological methods were compared with each other and with sequences from other Scottish patients.

Setting: Glenochil prison for men, central Scotland.

Subjects: Adult inmates and their possible contacts.

Results: Phylogenetic analysis of viral sequences in two different genomic regions showed that 13 of the 14 HIV positive prisoners had been infected from a common source. Previous research had shown that six of these had acquired their infection in Glenochil; molecular evidence suggests that more than double this number were infected while incarcerated. Virus from two long term HIV positive patients who were in the prison at the time of the outbreak but who were not identified in the original or subsequent surveys was sufficiently different to make it unlikely that they

were the source. A viral sequence from heterosexual transmission from one inmate showed the ability of these techniques to follow the infection through different routes of infection.

Conclusion: The number of prisoners infected with HIV during the 1993 outbreak within Glenochil prison was more than twice that previously shown. This shows the potential for the spread of bloodborne diseases within prisons by injecting drugs.

Introduction

Inmates who inject drugs in prison are at risk of acquiring HIV and other bloodborne infections.¹ Although several investigations into the prevalence of HIV infection have been conducted in correctional institutions,²⁻⁴ measuring the incidence of HIV transmission within prison is difficult because of problems in identifying the probable date of transmission. The first report to provide direct evidence of an outbreak of HIV occurring within a prison documented an HIV counselling and testing initiative of inmates from HM Prison Glenochil conducted at the end of June 1993; this exercise was precipitated by the reporting of eight symptomatic cases of acute hepatitis B and two diagnosed cases of primary infection with HIV between April and June 1993.⁵ Widespread drug injecting and needle sharing were also reported by the

prison doctor, and the possibility of an outbreak was recognised.

Of 378 inmates who were incarcerated during the survey period, 227 (60%) agreed to counselling and subsequently to an HIV test. This resulted in the identification of a further 12 cases of HIV infection. From known dates of imprisonment in Glenochil, the most recent negative result for HIV antibody, the presence of p24 antigenaemia, and clinical presentation of symptomatic primary HIV infection, it was concluded that eight inmates had acquired HIV infection while in prison. Of these eight, two could possibly have acquired HIV in another prison before transfer, thus for only six patients was there definite evidence of an infection acquired in Glenochil itself.⁵

HIV evolves at a rate estimated to be one million times faster than that of higher eukaryotes.⁶ This is because the rate of viral replication is rapid (about 10^{10} viral particles are generated daily)^{7,8} and the virus's reverse transcriptase enzyme, which is essential for replication, is error prone and lacks any proofreading capacity. Thus, any one infected person harbours a complex population of closely related but distinguishable viruses. Whereas this is a major problem for the design of therapeutic interventions, it can facilitate certain epidemiological investigations. An epidemiological link among infected people is likely if their viral nucleic acid sequences are similar and unlikely if they are different. To compare sequences from different people it is important to study the most appropriate region of the viral genome. The *env* gene evolves at such a rapid rate that an epidemiological link can be detected only if the interval between transmission and sampling is extremely short. In contrast, the *pol* gene is highly conserved in people not taking antiviral drugs and can be very similar among those who acquired their HIV infection from unrelated sources. The p17 region of the *gag* gene has been shown to be effective as a molecular epidemiological tool particularly when used in conjunction with the *env* gene.⁹

Molecular epidemiological analysis of nucleotide sequence data has been used in several investigations of HIV-1 transmission.¹⁰⁻¹⁴ Analysis of nucleotide sequence data established the occurrence and extent of an infection cluster associated with a dental practice in Florida¹⁰⁻¹⁵ and distinguished between two possible sources of infection in an investigation by the Maryland Department of Health.¹³ Similar methods were used here to conduct a molecular epidemiological investigation on samples from the HIV infected inmates identified at the time of the counselling and testing exercise.⁵ The aim of the study was to determine if all diagnosed cases were, as postulated, linked and if there had been a single or multiple source of infection.

Subjects and methods

Samples

Between June and August 1993 peripheral blood mononuclear cells were obtained from 13 of the 14 HIV positive inmates identified in the previously published survey (cases 1-7 and 9-14).⁵ In addition, a plasma sample was obtained from the other member of the cohort (case 8), from whom a follow up whole blood sample was unavailable. Despite repeated

attempts only a single viral sequence from this sample was generated. Case numbers used in this paper correspond with those published elsewhere.⁵

Peripheral blood mononuclear cells and plasma samples were obtained from two long term patients infected with HIV (S1 and S2, respectively) who had been identified by their general practitioners, who, in response to publicity surrounding the outbreak, realised that they had been in Glenochil during the "at risk" period (January to June 1993). They were therefore not considered part of the outbreak but as putative sources. Peripheral blood mononuclear cells were also obtained from the wife of one inmate (HC1), who seroconverted after heterosexual contact with her husband after his release from prison.

Sequencing

Proviral DNA and viral RNA was extracted from patients' peripheral blood mononuclear cells and plasma, respectively, and amplified by using nested polymerase chain reaction in both the V3/V4 region of the *env* gene and the p17 region of the *gag* gene.¹⁶⁻¹⁹ Direct sequencing of polymerase chain reaction products derived from single molecules was carried out by using an Applied Biosystems 373A automated sequencer.²⁰ Ideally, two sequences from each region from each person were generated but because of the quality of starting material this was not always possible.

Phylogenetic analyses

In the case of *gag* sequences a maximum likelihood phylogenetic tree, rooted by the inclusion of the subtype D isolate HIV-1_{ELI}, was obtained for duplicate sequences from cases 1, 2, 4-7, and 9-14 and single sequences from cases 3 and 8 and compared with duplicate sequences from the heterosexual contact of case 5 (HC1) and one putative source S1 (see fig 1). Single sequences within the homologous region of HIV, from seven injecting drug users, five haemophilic subjects, three heterosexual contacts of injecting drug users, all of whom were Scottish and not known to be related for HIV transmission, and 10 unrelated subtype B isolates obtained from international databases were included for comparison.

A similar tree was used to compare a 246 base pair region of the *env* gene (see fig 2). Duplicate sequences from Glenochil cases 1, 2, 4-7, and 9-13, a single sequence from case 3, and duplicate sequences from the sexual contact (HC1) and both putative sources S1 and S2 were aligned with the homologous region from 14 haemophilic subjects and five injecting drug users and heterosexual contacts, all of whom were Scottish and not known to be related for HIV transmission, and 10 unrelated subtype B isolates from international databases.

Results

Analysis of p17 *gag* sequences

This analysis grouped 13 of the 14 Glenochil inmates into a single cluster; indeed 16 sequences from nine different people had identical sequences over the 350 base pairs used for the comparison (fig 1). The heterosexual contact of case 5 (HC1) clearly groups within the main cohort whereas the sequence from putative

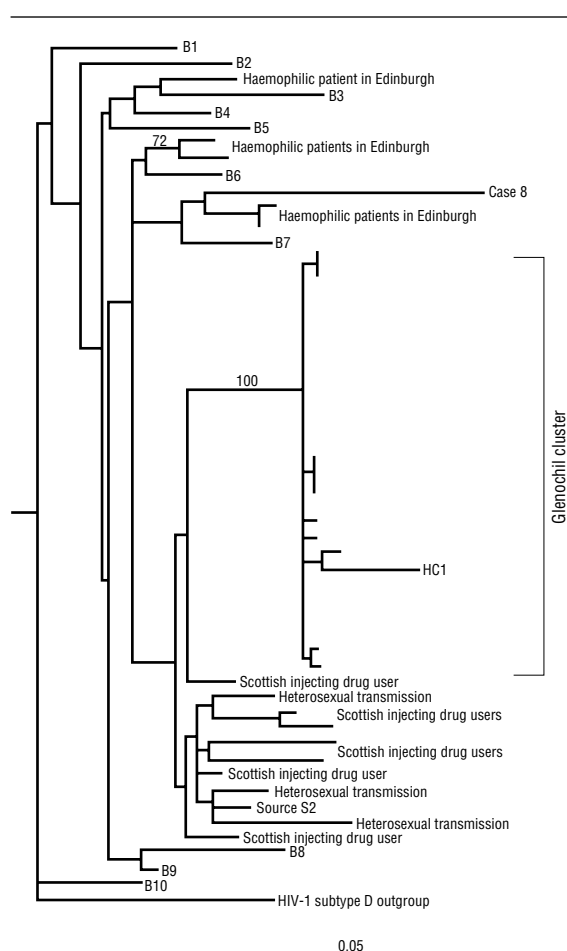


Fig 1 Maximum likelihood phylogenetic tree of gag sequences from Glenochil cohort compared with related and unrelated isolates. B1-11=unrelated HIV-1 B subtype isolates obtained from international databases (1=JRCSE, 2=HAN, 3=RF, 4=CDC4, 5=OYI, 6=JH3, 7=NY5, 8=MN, 9=LAI, 10=SF2, 11=SC). Numbers refer to bootstrap replicate values greater than 70%; scales denote branch length (%)

source S2 groups with other Scottish drug users unrelated to the outbreak. The single sequence obtained from the 14th Glenochil inmate (case 8) was distinct. Analysis in a wider background showed that it grouped among sequences found in several homosexual men from Scottish cities (A J Leigh Brown *et al*, unpublished data).

Env gene sequences

Figure 2 shows the maximum likelihood phylogenetic tree that illustrates that all of the 12 Glenochil patients from whom sequences were obtained in this particular region not only fall into a single cluster, supporting the gag data, but also have no specific association with either of the potential sources of the infection.

Statistical assessment of phylogenetic analysis

The phylogenetic trees for both gag and env were tested by bootstrap resampling and results presented as a percentage. The bootstrap procedure identifies clusters of sequences which are still present after the nucleotide dataset has been resampled. Clusters that are present in 70% or more bootstrap samples are considered to be well supported.¹⁵ Every significant group—that is, supported in 70% or more samples—is identified in the figures. With the single exception of

case 8 the entire Glenochil cohort has a bootstrap support of 100% in the p17 gag tree and of 85% in the V3 env region tree. The only other supported clusters (apart from multiple sequences from the same patient) were those which have previously been described for haemophilic subjects in Edinburgh.¹⁹

A more specific evaluation was required to test the possibility of linkage between the major Glenochil cluster of sequences and either of the putative sources. This was done by constructing alternative trees in which either of these two patients was grouped with the cohort and testing the significance of the difference by a maximum likelihood ratio test.²¹ Three rearrangements of the V3 tree data were tested by artificially altering the tree to see if the result was a significantly worse fit. Versions that either grouped any individual members of the main Glenochil cluster with other Scottish drug users or that introduced putative source S2 into the main Glenochil cluster were significantly less likely ($P < 0.05$) than that shown in figure 2. The rearrangement that grouped the other putative source (S1) with this group, however, was not significantly less likely than the tree shown.

Similar analyses on the gag data were performed. Both the inclusion of sequences from either case 8 or putative source S2 into the main Glenochil cluster resulted in a tree that was significantly less likely than the one shown ($P < 0.05$).

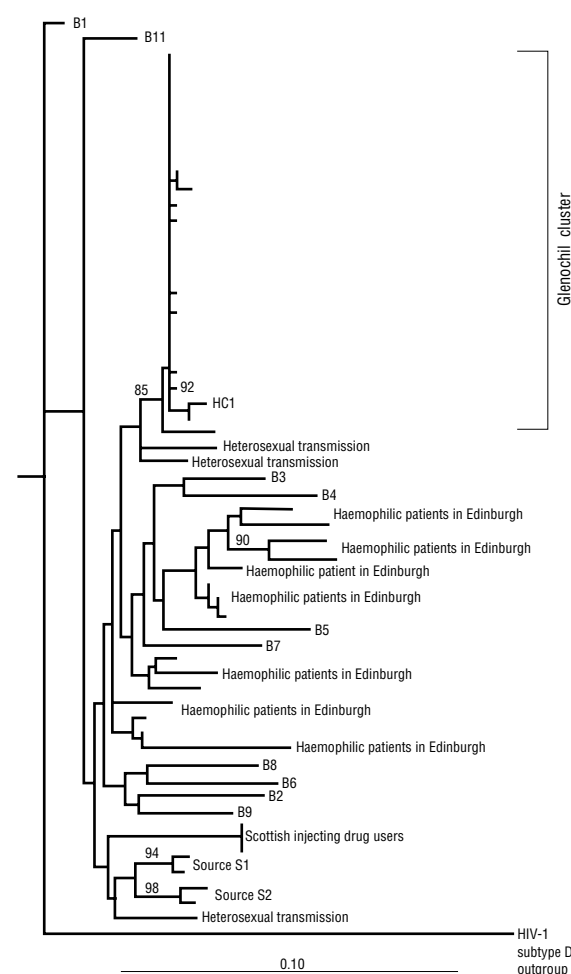


Fig 2 Maximum likelihood phylogenetic tree of env sequences from Glenochil cohort compared with related and unrelated isolates (see legend to figure 1 for detailed explanation)

Summary of results

The similarity between the viral sequences from different people is remarkable. In the gag region the average divergence within the cluster of 13 was 0.2%. This compares with an average divergence between Scottish drug users of 5.4% ($P < 0.001$) and subtype B viruses generally of 6.7%. Even within the V3 region of the envelope gene where the average divergence within B subtypes and Scottish drug users is higher at 11.4% and 8.6%, respectively, divergence within the cohort is only 0.65%. Indeed, the values for the cohort are comparable with or even lower than those that would be expected from sequential samples obtained from the same person. As six of this group had previously been shown to have acquired their infection while in Glenochil prison,⁵ it follows that all 13 were part of the same epidemiological cluster. The sequence from the 14th patient (case 8) is significantly different. The two putative sources of infection (S1 and S2), identified serendipitously by local general practitioners and not by prison surveys, were known to have acquired their infection before the outbreak in Glenochil and are therefore not considered as part of this particular cohort. The sequences from these two people were sufficiently different from the cluster of 13 for us to discount linkage, although only in the case of S2 was this significant. The linkage of sequences from the heterosexual contact HC1 showed a significant linkage with the cluster of 13.

Discussion

HIV in prisons

The prevalence of HIV infections in different prisons within and across countries can vary considerably. Prevalence has ranged from none in a young male offenders institution in Scotland³ to 33.6% in an adult prison in Catalonia²² and to over 50% in a female correctional facility in New York City.² Most HIV infected inmates probably acquired their infection through injecting drug use, very little being ascribed to homosexual contact.²³ In Australia an estimated half of male injecting drug users have at some time been imprisoned,⁴ and several reports have indicated that not only do a high proportion of drug users continue to inject and share injecting equipment while incarcerated but some inject for the first time while in prison.^{1 24 25} It is unknown but could be expected that outbreaks such as that reported in Glenochil prison are more widespread. Several surveys in Scottish prisons have indicated that the medical authorities have underestimated the number of HIV positive people in their prison by about 25%.^{1 26} For example, the initial survey of Glenochil prison in June 1993⁵ identified 14 HIV positive prisoners, whereas a follow up study 12 months later estimated that up to 20 inmates had been part of the outbreak.²⁷

Application of molecular technology

Before this investigation the combination of conventional serological data and dates of prison entry had been used to show that eight out of the 14 HIV positive inmates of Glenochil prison had acquired their infection while incarcerated, although two of these could have been infected in another prison before transfer. By applying molecular techniques we have

shown that 13 of the 14 HIV positive subjects were infected with an almost identical virus. This finding strongly suggests that not only were these men infected while in Glenochil prison but also the infection came from a single source. As the two inmates previously known to be infected (S1 and S2) had viral sequences that were unrelated to those seen in the cohort, we conclude that the source of the infection was either an unidentified Glenochil inmate or one of the 13 cases who acquired his infection outside the prison just before being incarcerated there early in 1993. Infection was then probably transmitted by this single source directly or indirectly along a chain to either 12 or 13 inmates over a short period of time. The rapid spread of HIV among the cohort probably occurred while concentrations of circulating virus were at their highest in each subject during the interval between infection and seroconversion.²⁸

The 14th person identified by the survey (case 8) was clearly infected with a different virus, with an average sequence divergence from the rest of the cohort of 12.4%. Only a single sample, which had shown an early banding pattern on western blot characteristic of seroconversion, was available for this patient. In the absence of a follow up sample it seems that he was infected at the same time as the others but not from the same source. Indeed, although no sex between men was reported from the Glenochil survey, analysis within a more extensive dataset shows that the sequence from case 8 is most closely related to viral sequences obtained from homosexual men, suggesting a different source of infection. In this context the Glenochil cohort will refer to the 13 patients infected with a similar viral strain.

Public health implications

The application of molecular techniques to trace the origin or chain of an outbreak of infection is not new and has been particularly useful in the context of food poisoning. For HIV the benefits of viral sequencing may also be considerable. Before 1993 no outbreak of HIV within a prison had been reported. While there was much indirect evidence to suggest that epidemics had occurred in prisons, the absence of proof made it difficult for those working in the specialties of HIV and AIDS to convince prison officials and authorities that preventive measures needed to be implemented.

The finding that at least 13 prisoners definitely acquired their infection inside Glenochil prison was the catalyst for the introduction of a wide range of harm reduction measures for people who inject in prisons throughout Scotland. These include the provision of bleach tablets for sterilising injecting equipment; a methadone detoxification programme for inmates; the availability of hepatitis B vaccine; increased training for prison officers; and improved access to drug harm minimisation counselling for prisoners. Thus the public health implications of this molecular investigation have already been considerable. As drug injecting inside prison is a global problem, it is hoped that the findings of this study will have far reaching consequences.

The Glenochil cohort strain belongs to the subtype B category, which is the predominant strain in Europe and North America. Non-B subtypes, which are prevalent in the developing world, however, are being

Key messages

- Originally, standard serological studies established that six out of 14 HIV positive people had acquired their infection in Glenochil prison in 1993
- A subsequent survey estimated that up to 20 prisoners were HIV positive at that time
- Molecular linkage techniques showed that 13 of the original 14 infected men had viral sequences similar enough to indicate that one source of infection was common to all
- Molecular techniques provide a powerful epidemiological tool either to link or to distance infections with HIV
- Injecting drug use is a potentially explosive health care problem in prisons

detected increasingly in the United Kingdom as a consequence of travel and mixing of populations. Of particular concern is HIV subtype E, which is highly prevalent among heterosexuals in South East Asia and for which there is laboratory evidence of properties that suggest that it may be transmitted heterosexually more efficiently than B subtype strains (A J Leigh Brown *et al*, unpublished data). Already the public health laboratory service in England has identified several different viral subtypes.²⁹ It is thus essential that surveillance of HIV infection includes not only data on numbers of prevalent cases but also information on strain subtype. Such monitoring would identify the entry of new strains into the population from abroad, the occurrence of which could have a major impact on the dynamics of HIV spread locally.

Future studies

Further work that has stemmed from the findings of this study is under way. The Glenochil cohort is unique; all members are of similar age, the same sex, ethnic group, and risk category, and all were infected with the same virus at the same time. This feature affords the opportunity to study progression of HIV disease in a cohort for which viral as well as host factors are controlled. Although the cohort comprises 13 cases, there could be more. In July 1994 a voluntary anonymous prevalence study of HIV infection among inmates of Glenochil prison estimated that 20 had been infected during January to June 1993. It is therefore likely that some cases have yet to be diagnosed or associated with the Glenochil outbreak. Accordingly, approval has been obtained to link the prison register for the period January to June 1993 with the HIV register held at the Scottish Centre for Infection and Environmental Health; the latter contains details on all new laboratory diagnoses in Scotland. If a match is identified efforts will be made, on condition of consent, to verify the association through an interview with the subject and molecular investigations being conducted on his blood to compare his strain with that of the original cohort.

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Conflict of interest: None.

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Residential and occupational exposure to sunlight and mortality from non-Hodgkin's lymphoma: composite (threefold) case-control study

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Abstract

Objective: To determine whether non-Hodgkin's lymphoma mortality is associated with sunlight exposure.

Design: Three case-control studies based on death certificates of non-Hodgkin's lymphoma, melanoma, and skin cancer mortality examining associations with potential sunlight exposure from residence and occupation.

Setting: 24 states in the United States.

Subjects: All cases were deaths from non-Hodgkin's lymphoma, melanoma, and non-melanotic skin cancer between 1984 and 1991. Two age, sex, and race frequency matched controls per case were selected from non-cancer deaths.

Main outcome measures: Odds ratios for non-Hodgkin's lymphoma, melanoma, and skin cancer from residential and occupational sunlight exposure adjusted for age, sex, race, socioeconomic status, and farming occupation.

Results: Non-Hodgkin's lymphoma mortality was not positively associated with sunlight exposure based on residence. Both melanoma and skin cancer were positively associated with residential sunlight exposure. Adjusted odds ratios for residing in states with the highest sunlight exposure were 0.83 (95% confidence interval 0.81 to 0.86) for non-Hodgkin's lymphoma, 1.12 (1.06 to 1.19) for melanoma, and 1.30 (1.18 to 1.43) for skin cancer. In addition, non-Hodgkin's lymphoma mortality was not positively associated with occupational sunlight exposure (odds ratio 0.88; 0.81 to 0.96). Skin cancer was slightly positively associated with occupational sunlight exposure (1.14; 0.96 to 1.36).

Conclusions: Unlike skin cancer and to some extent melanoma, non-Hodgkin's lymphoma mortality was not positively associated with exposure to sunlight. The findings do not therefore support the hypothesis that sunlight exposure contributes to the rising rates of non-Hodgkin's lymphoma.

Introduction

Incidence and death rates for non-Hodgkin's lymphoma have risen rapidly in recent decades throughout the world.¹ Neither changes in diagnostic practices nor known and suspected risk factors seem to account fully for the increases.² Several investigators have hypothesised that increased exposure to sunlight may have contributed to the rising rates of non-Hodgkin's lymphoma.³⁻⁸ They note that melanoma and other skin cancer rates have also risen rapidly worldwide,¹ that non-Hodgkin's lymphoma and skin cancer are associated in individual patients,^{4,6} and that ultraviolet stimulation is experimentally immunosuppressive.^{3,7} Ecological studies that have explored this hypothesis have had con-

flicting results.^{3,7-9} None, however, examined sources of sunlight exposure other than residence.

We conducted a population based case-control study of non-Hodgkin's lymphoma mortality in the United States. As an improvement over ecological studies, we assessed potential sunlight exposure from both the occupational and the residential records on the death certificate. The findings for non-Hodgkin's lymphoma were compared with findings for melanoma and non-melanotic skin cancer, two diseases regarded as causally linked to sunlight exposure.¹⁰

Methods

Since 1984 the American National Cancer Institute and the National Institute for Occupational Safety and Health have supported the creation of a 24 state mortality database, which includes coding of occupation and other information from death certificates, as described by Figs *et al.*¹¹ Cases included all deaths between 1984 and 1991 from non-Hodgkin's lymphoma (International Classification of Diseases, ninth revision (ICD-9), codes 200 and 202 (excluding 202.2-202.6)), melanoma (code 172), and non-melanotic skin cancer (codes 173, 154.3, and 187.7) in people aged 20 or over identified as white or African American. Two controls per case were selected from non-cancer deaths in the database and matched for frequency by sex, race, and five year age group.

Potential sunlight exposure was assessed by residence and usual occupation recorded on the death certificate. The annual mean daily solar radiation (which includes ultraviolet and visible light) for the state of residence at death and state at birth were obtained from United States Weather Bureau data.¹² Each state was characterised at one of three levels based on the predominant solar radiation contour crossing the state. The 24 states reflected all regions of the country (see table 1).

Occupations were classified by an industrial hygienist (MD) into four categories: indoor work, work that combined indoor and outdoor exposure, outdoor work by non-farmers, and farming. Farmers were categorised separately because some studies have suggested that farmers may be at increased risk of non-Hodgkin's lymphoma due to exposure to pesticides, a potential confounder for which we could not otherwise control.¹³ Occupation was also used to create an index of socioeconomic status, with five levels based on a method of scoring devised by Green.¹⁴ Homemakers, retirees, and students were not assigned a socioeconomic level but were controlled for separately. Cases and controls with no identified occupation on the death certificate (about 4%) were excluded from analysis.

The multivariate model included age, sex, race, residential sunlight exposure, occupational sunlight

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Table 1 Characteristics of non-Hodgkin's lymphoma, melanoma, and non-melanotic skin cancer cases and controls.† Data expressed as numbers (percentages) of subjects

	Non-Hodgkin's lymphoma		Melanoma		Skin cancer	
	Cases (n=33 407; 34%)	Controls (n=65 843; 66%)	Cases (n=12 156; 34%)	Controls (n=23 845; 66%)	Cases (n=4619; 33%)	Controls (n=9170; 67%)
Age (years):						
20-44	2 320 (7)	4 413 (7)	2 290 (19)	4 361 (18)	270 (6)	526 (6)
45-54	2 587 (8)	4 994 (8)	1 753 (14)	3 378 (14)	401 (9)	798 (9)
55-64	5 646 (17)	11 089 (17)	2 405 (20)	4 742 (20)	912 (20)	1803 (20)
65-74	9 885 (30)	19 590 (30)	2 753 (23)	5 474 (23)	1163 (25)	2302 (25)
≥75	12 969 (39)	25 757 (39)	2 955 (24)	5 890 (25)	1873 (41)	3741 (41)
Sex:						
Female	16 609 (50)	32 822 (50)	4 900 (40)	9 590 (40)	1566 (34)	3125 (34)
Male	16 798 (50)	33 021 (50)	7 256 (60)	14 255 (60)	3053 (66)	6045 (66)
Race:						
Black	1 818 (5)	3 535 (5)	208 (2)	400 (2)	410 (9)	810 (9)
White	31 589 (95)	62 308 (95)	11 948 (98)	23 445 (98)	4209 (91)	8360 (91)
Pigmentation:						
Fair‡	1 806 (5)	3 782 (6)	691 (6)	1 346 (6)	232 (5)	500 (5)
Other white	29 783 (89)	58 526 (89)	11 252 (93)	22 099 (93)	3977 (86)	7860 (86)
Black	1 818 (5)	3 535 (5)	208 (2)	400 (2)	410 (9)	810 (9)
Residence§:						
Low sun	11 553 (35)	21 244 (32)	3 646 (30)	7 613 (32)	1330 (29)	2972 (32)
Moderate sun	13 529 (41)	26 334 (40)	4 792 (39)	9 384 (39)	1839 (40)	3692 (40)
High sun	8 325 (25)	18 265 (28)	3 718 (31)	6 848 (29)	1450 (31)	2506 (27)
Birthplace :						
Low sun	11 000 (33)	20 496 (31)	3 797 (31)	7 626 (32)	1252 (27)	2757 (30)
Moderate sun	14 290 (44)	27 861 (42)	5 135 (42)	10 023 (42)	2007 (43)	3885 (42)
High sun	6 863 (21)	15 245 (23)	2 870 (24)	5 478 (23)	1218 (26)	2147 (23)
Occupation:						
Indoor	17 054 (51)	31 125 (47)	6 574 (54)	11 734 (49)	2225 (48)	4393 (48)
Mixed	14 081 (42)	29 502 (45)	4 723 (39)	10 166 (43)	1849 (40)	3793 (41)
Outdoor (non-farmer)	867 (3)	2 302 (4)	406 (3)	993 (4)	250 (5)	441 (5)
Farmer	1 405 (4)	2 914 (4)	453 (4)	952 (4)	295 (6)	543 (6)
Socioeconomic status¶:						
1 Low	3 653 (11)	8 917 (14)	1 263 (10)	3 151 (13)	799 (17)	1530 (17)
2	4 889 (15)	11 055 (17)	1 774 (15)	4 497 (19)	821 (18)	1715 (19)
3	9 771 (29)	17 815 (27)	3 910 (32)	7 015 (29)	1320 (29)	2612 (28)
4	5 177 (15)	8 001 (12)	2 153 (18)	3 245 (14)	575 (12)	1148 (13)
5 High	1 449 (4)	1 954 (3)	754 (6)	878 (4)	187 (4)	311 (3)

†Ratio of controls to cases was slightly less than 2.0 owing to exclusion of subjects with no occupational information.

‡Subjects were characterised as fair skinned if they were white and their national origins were identified as British, Irish, German, Scandinavian, Polish, or other northern European.

§Levels of sun exposure were categorised based on annual mean daily solar radiation reported by Garland *et al*¹² for state reported as residence at time of death. This was sometimes outside the 24 states in which deaths occurred. "Low" included the following states and other areas: Alaska, Connecticut, *Maine*, Massachusetts, Michigan, Minnesota, *New Hampshire*, New York, *Ohio*, Oregon, Pennsylvania, *Rhode Island*, Vermont, Washington, Wisconsin, Canada. "Moderate" included Arkansas, Delaware, District of Columbia, *Idaho*, Illinois, *Indiana*, Iowa, *Kansas*, Kentucky, Maryland, *Missouri*, Montana, *Nebraska*, *New Jersey*, *North Carolina*, North Dakota, South Carolina, *Tennessee*, Virginia, and *West Virginia*. "High" included Alabama, Arizona, California, *Colorado*, Florida, *Georgia*, Hawaii, Louisiana, Mississippi, *Nevada*, *New Mexico*, *Oklahoma*, *South Carolina*, Texas, *Utah*, Wyoming, Puerto Rico, Virgin Islands, Guam, Cuba, and Mexico. (States in bold are the 24 states from the mortality database.)

||Levels of sun exposure were categorised as above for state identified as state of birth. Cumulative percentage is less than 100 because birthplace could not be identified in a few cases and controls.

¶Cumulative percentage is less than 100 because socioeconomic status could not be identified for retirees, homemakers, and students.

exposure, and socioeconomic status for the entire population, and was analysed by race and sex groups and by age (<45 years, ≥45 years). Birthplace, which was highly correlated with residence at death, was excluded from the model. The effects of skin pigmentation were assessed by classifying subjects based on national origin and race as recorded on the death certificate.

The measure of association was the mortality odds ratio and 95% confidence interval derived by standard logistic regression methods in SAS.¹⁵

Results

Table 1 gives the numbers of cases and controls for non-Hodgkin's lymphoma, melanoma, and non-melanotic skin cancer deaths by age, sex, race, skin

pigmentation, residence, birthplace, occupational category, and socioeconomic status. Roughly a quarter to one third of subjects lived in states with the highest levels of sunlight. Around 3-5% had outdoor non-farming jobs.

Table 2 gives the odds ratios for non-Hodgkin's lymphoma, melanoma, and non-melanotic skin cancer mortality adjusted for age, sex, race, residence, occupational sunlight exposure, and socioeconomic status. For non-Hodgkin's lymphoma the adjusted odds ratios declined significantly with increasing exposure to sunlight based on residence and occupational category. In contrast, the adjusted odds ratios for melanoma and skin cancer and residential exposure increased, the risk of skin cancer rising significantly to 1.30 among subjects who resided in states with the highest sunlight exposure.

The risk of non-farming occupational sunlight exposure for skin cancer was slightly but not significantly raised in outdoor workers (odds ratio 1.14; table 2). In melanoma, however, there was no apparent pattern for non-farming occupational sunlight exposure. Farmers were at significantly increased risk compared with indoor workers for non-Hodgkin's lymphoma and melanoma but not skin cancer. Socioeconomic status increased the association with non-Hodgkin's lymphoma and melanoma but generally not with skin cancer. Similar results were obtained in the crude analysis, with the exception of the odds ratio for farming; this was significantly decreased for non-Hodgkin's lymphoma and melanoma.

The negative residential associations identified for non-Hodgkin's lymphoma in the total population were also seen in the multivariate model in the sex, race, and age subpopulations. The deficit of non-Hodgkin's lymphoma risk associated with residential sunlight exposure was most pronounced in subjects aged 44 years or less (highest versus lowest sunlight region: odds ratio 0.69; 95% confidence interval 0.61 to 0.79). The odds ratio for occupational exposure among men reflected that of the total population. Among women the odds ratios were raised, though not significantly so.

The risk of melanoma and skin cancer increased with residential exposure among white people and subjects aged over 44. No pattern was evident for African Americans. There was also no clear pattern for melanoma or skin cancer risk associated with occupational exposure across the sex, race, or age groups, except that risk of skin cancer increased among subjects aged over 44.

Increased potential sunlight exposure was associated with declining non-Hodgkin's lymphoma risk in each residential area and for each occupational category, the greatest decline occurring among outdoor workers in areas receiving the most sunlight (table 3). The effect was strongest in younger people, among whom the odds ratio declined to 0.44 (0.28 to 0.67) in subjects in occupations and states with the highest sunlight exposure. The risks for skin cancer generally increased with potential residential or occupational sunlight exposure. With melanoma, however, the risk from residential exposure did not increase among outdoor workers; nor did the risk increase with occupational sun exposure, except among subjects with low residential exposure.

Odds ratios for non-Hodgkin's lymphoma increased with skin pigmentation from 1.0 among subjects with northern European ancestry to 1.10 (1.04 to 1.17) among other white groups and 1.26 (1.16 to 1.37) among African Americans. There was no significant association with melanoma or skin cancer. Among subjects with fair pigmentation the odds ratio for non-Hodgkin's lymphoma in the highest sunlight region was 0.92 (0.80 to 1.06). In this group the risks associated with this exposure were raised for melanoma (odds ratio 1.44; 1.15 to 1.79) and skin cancer (1.56; 1.06 to 2.29).

Discussion

This study found no evidence of an excess risk of non-Hodgkin's lymphoma mortality associated with potential sunlight exposure. The results generally showed

Table 2 Odds ratios (95% confidence intervals) for non-Hodgkin's lymphoma, melanoma, and non-melanotic skin cancer mortality associated with indicators of sunlight exposure and socioeconomic status, adjusted for age, sex, race, and other factors†

	Non-Hodgkin's lymphoma	Melanoma	Skin cancer
Residence‡:			
Low sun	1.0	1.0	1.0
Moderate sun	0.95 (0.92 to 0.98)	1.08 (1.02 to 1.13)	1.12 (1.02 to 1.22)
High sun	0.83 (0.81 to 0.86)	1.12 (1.06 to 1.19)	1.30 (1.18 to 1.43)
Occupation:			
Indoor	1.0	1.0	1.0
Mixed	0.95 (0.91 to 0.99)	0.92 (0.87 to 0.98)	0.95 (0.85 to 1.05)
Outdoor (non-farmer)	0.88 (0.81 to 0.96)	0.99 (0.87 to 1.12)	1.14 (0.96 to 1.36)
Farmer	1.31 (1.21 to 1.42)	1.31 (1.14 to 1.52)	1.08 (0.89 to 1.31)
Socioeconomic status:			
1 Low	1.0	1.0	1.0
2	1.21 (1.13 to 1.28)	1.11 (1.00 to 1.23)	0.97 (0.84 to 1.12)
3	1.49 (1.40 to 1.57)	1.55 (1.41 to 1.71)	1.03 (0.90 to 1.18)
4	1.73 (1.62 to 1.84)	1.81 (1.63 to 2.00)	1.01 (0.86 to 1.18)
5 High	2.02 (1.86 to 2.20)	2.39 (2.10 to 2.72)	1.22 (0.98 to 1.52)

† Odds ratios were calculated from logistic regression, adjusted for age (categorised as in table 1), sex, race, residence, occupational sun exposure, and socioeconomic status.

‡ See table 1.

Table 3 Odds ratios (95% confidence intervals) for non-Hodgkin's lymphoma, melanoma, and non-melanotic skin cancer mortality by occupational sunlight exposure and residential sunlight exposure, adjusted for age, sex, race, socioeconomic status, and farming status†

Occupational exposure	Residence‡		
	Low sun	Moderate sun	High sun
Non-Hodgkin's lymphoma			
Indoor	1.0	1.0 (0.96 to 1.04)	0.86 (0.82 to 0.90)
Outdoor (non-farmer)	0.89 (0.77 to 1.03)	0.87 (0.76 to 0.99)	0.74 (0.64 to 0.86)
Melanoma			
Indoor	1.0	1.10 (1.03 to 1.19)	1.18 (1.09 to 1.27)
Outdoor (non-farmer)	1.22 (0.99 to 1.50)	1.05 (0.86 to 1.29)	0.96 (0.76 to 1.20)
Skin cancer			
Indoor	1.0	1.06 (0.94 to 1.19)	1.28 (1.12 to 1.46)
Outdoor (non-farmer)	0.98 (0.71 to 1.34)	1.42 (1.08 to 1.84)	1.36 (1.01 to 1.83)

† Odds ratios were calculated from logistic regression; adjustments for age were based on age categories in table 1.

‡ See table 1.

decreased risk among people with the heaviest exposure. Though the methods used were crude, they produced associations between sunlight exposure and the risk of melanoma and skin cancer mortality. Most other research on non-Hodgkin's lymphoma and sunlight has been limited to ecological data with exposure potential determined by residence alone. This analytical study improved exposure ascertainment by using individual data on occupation, state of birth, socioeconomic status, skin pigmentation, and residence.

Associations with exposure to sunlight

The incidence of skin cancer is thought to be directly associated with cumulative sun exposure.^{10 16} Generally, studies of skin cancer incidence and total sun exposure have found associations with odds ratios between 1.2 and 11.0.¹⁷ Consistent with this, we found positive associations between skin cancer mortality and residential and occupational surrogates for exposure to sunlight exposure. In contrast, melanoma was not as closely associated with our indicators of sunlight exposure. The relation of melanoma to sunlight, however, is more complex, with age of exposure^{3 10} and intermit-

tent intense exposure^{10 18 20} thought to have a role. Studies of melanoma incidence and residential or overall sun exposure have reported significant associations with odds ratios or relative risk ratios of about 1.7.^{16 18} In this study residential sunlight exposure at the times of death and birth was associated with melanoma.

Occupational sunlight exposure, however, was not associated with melanoma. The absence of an overall occupational association seems to reflect varied occupational associations among residential regions. In both the moderate and high sunlight regions outdoor work was associated with lower risk than indoor work, which might be expected if melanoma is more a function of intermittent exposure than continuous exposure. Occupational studies of melanoma risk and outdoor exposure present divergent findings, studies indicating no association with outdoor work¹⁹ or that outdoor work may protect against melanoma.^{18 20}

In marked contrast with the associations with melanoma and skin cancer, we found that both residential and occupational sunlight exposure were inversely associated with non-Hodgkin's lymphoma and that these negative associations characterised most of the sex, race, and age subpopulations. Moreover, risk declined consistently across residential exposure categories as occupational exposure increased and across occupational categories as residential exposure increased. This was true even when the exposure might be intermittent, as in the case of indoor workers living in high sunlight exposure areas.

The stronger negative association with sunlight exposure among younger people further argues against a positive causal role for sunlight exposure in non-Hodgkin's lymphoma. In younger people there is less likely to be misclassification of exposure because occupations and residences recorded at death are less commonly succeeded by a retirement period or preceded by a retirement move.

In support of the hypothesis that sunlight contributes to the incidence of non-Hodgkin's lymphoma, Adami cited the observed increased risk of non-Hodgkin's lymphoma in outdoor workers.²¹ Particular outdoor occupations, including farming, forestry, and fishing, have been associated with increased risk of non-Hodgkin's lymphoma.²² We examined the collective risk of about 40 outdoor jobs other than farming, as well as farming, an occupational exposure that potentially posed particular risks. Our results showed a negative association for outdoor work generally whereas, as expected, the odds ratio for farming was raised.

Other findings in our study supported an association between sunlight exposure and skin cancer and melanoma but not non-Hodgkin's lymphoma. Both melanoma^{23 24} and skin cancer¹⁰ have been associated with fair skin. If sunlight plays a part in non-Hodgkin's lymphoma skin pigmentation may similarly affect susceptibility. Thus the lower incidence of non-Hodgkin's lymphoma among African Americans has been cited as supporting the sunlight-non-Hodgkin's lymphoma hypothesis.²¹ The reported lower incidence in African Americans in the United States, however, derives from cancer registry data and is not adjusted for socioeconomic status.²⁵ When we control-

led for socioeconomic status the risks in African Americans exceeded those in people likely to have fair pigmentation and other white subjects. Moreover, there was no association between non-Hodgkin's lymphoma and residential sunlight exposure among people with fair pigmentation. In contrast, residential sunlight exposure was strongly associated with skin cancer and melanoma in this group.

Assessing exposure

There were limitations in the assessments of exposure in this study. Characterisation of residential sunlight exposure is subject to many sources of potential misclassification bias. Solar radiation contours were based on data from weather stations that could not measure all places and all conditions. The usual occupation entered on a death certificate may reflect most recent occupation, not a lifetime occupational history. Furthermore, residential exposure was based on state of residence at death, not lifetime residential history. None the less, depending on the region, between about 70% and 80% of subjects were born in the same exposure regions that they resided in at death. Moreover, to the extent that such misclassifications are made non-differentially the resulting bias is likely to dilute associations. This suggests that the true association with non-Hodgkin's lymphoma could be a greater deficit than observed here.

Socioeconomic and cultural differences

Socioeconomic status based on usual occupation at the time of death also could not account for lifetime occupational histories, education, or income. Yet the associations observed with socioeconomic status support the validity of the classification system. Socioeconomic status as categorised in this model showed a dose-response relation with the risk of melanoma, which is consistent with the findings of several studies.^{24 26 27} Socioeconomic status also seemed related to non-Hodgkin's lymphoma risk in our study, an association noted in some but not all populations.²⁵

We considered the possibility that cultural differences between states in the northern and southern latitudes of the United States would increase the HIV related non-Hodgkin's lymphoma mortality in the north and thus confound observed associations. That we did not include death certificates from California, New York, Florida, or Texas—four states with the highest percentage of AIDS cases²⁸—limits substantially potential confounding by HIV related non-Hodgkin's lymphoma mortality. Moreover, such confounding if present would not seem to account for the negative association between residential sunlight and non-Hodgkin's lymphoma in view of the fact that the negative association also characterised the subpopulations of white men, white women, black men, black women, young people, and old people.

The associations explored in this study between sunlight indicators and non-Hodgkin's lymphoma were virtually all negative. That they did not resemble the associations observed between sunlight and melanoma, and particularly sunlight and non-melanotic skin cancer, argues against sunlight as a strong explanatory factor in the increased incidence of non-Hodgkin's lymphoma. This analysis cannot, however, rule out a complex role for sunlight in the

Key messages

- The incidence of non-Hodgkin's lymphoma has risen rapidly in recent decades throughout much of the world
- Several investigators have hypothesised that increased exposure to sunlight may contribute to this rising incidence
- A study in the United States found that sunlight exposure based on residence and occupation was not positively associated with non-Hodgkin's lymphoma
- The study found no evidence to support the hypothesis that sunlight contributes to the rising rates of non-Hodgkin's lymphoma

aetiology of non-Hodgkin's lymphoma. There may, for example, be unsuspected environmental agents that are both correlated with northern latitudes of the United States and potent causal agents for non-Hodgkin's lymphoma and which mask a positive but weaker association with sunlight. It is possible that such factors are correlated with different latitudes in different countries, thus explaining the varied findings of ecological studies of latitude and non-Hodgkin's lymphoma.⁷⁻⁹ Our finding that indoor work and regions of low sunlight exposure increase, not decrease, the risks of non-Hodgkin's lymphoma needs to be confirmed, and the elusive agent or agents accounting for this increase need to be identified.

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Transfer of adults between intensive care units in the United Kingdom: postal survey

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In 1986, at least 10 000 seriously ill patients in the United Kingdom required secondary transfer to adult intensive care units in other hospitals.¹ Although 75 of 181 (41%) intensive care consultants were dissatisfied with transfer arrangements, only 10% (number not provided) ever refused a request for transfer. The establishment of dedicated regional transport services was recommended. It has also been recommended that patients should be retrieved by teams from receiving intensive care units, and that local capabilities be maintained

for urgent transfer of patients with head injuries.^{2,3} We reviewed current secondary transfer facilities and numbers and established the main indications for transfers.

Methods and results

Late in 1994 we surveyed 278 general or mixed intensive care units in the United Kingdom by postal questionnaire; 198 (71%) responded. The mean annual admission rate to intensive care units was 353 (range

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40-1540) patients, and annually an average of 23 patients were transported to each unit. The most frequently quoted reasons (not mutually exclusive) for such transfers were lack of intensive care beds (125; 63%) and of renal support services (45; 23%) in referring hospitals. Only 25 intensive care units admitted more than 40 transferred patients a year.

On average, 19 patients were transported from each unit each year. The most common indications for these transfers were referral for neurosurgical care (109; 55%), lack of beds in the intensive care unit (87; 44%), and lack of renal support services (54; 27%). Only 12 intensive care units transferred more than 40 patients a year to another hospital.

Staff and equipment for transfers were available in 191 (97%) hospitals. The 24 (12%) intensive care units which provided retrieval teams received on average 55 transferred patients a year. Only two hospitals provided "regional" transport teams. Table 1 shows equipment and staff resources. Eighty two (41%) respondents considered that arrangements for transfer were unsatisfactory. Despite this, only 19 (10%) stated that lack of facilities ever prevented patient transfer.

Comment

On the basis of data from our survey and an audit of admissions to a regional neurosurgical unit⁴ we estimate that the number of critically ill patients requiring secondary transport to adult intensive care units in Britain in 1994 exceeded 11 000. This estimate correlates well with that of the 1986 survey. Targeted provision of staffed beds and renal support services in existing general intensive care units would reduce the number of transfers. Conversely, regionalisation of specialist intensive care services may increase transfers unless there are fewer hospitals with small intensive care units and accident and emergency departments.

Most patients were transferred by staff from referring hospitals. Most medical escorts were unsupervised junior trainees in anaesthetics, each likely to experience few transfers. Transportation by doctors lacking suitable experience may result in a higher incidence of life threatening complications,⁵ especially as recommended monitoring is not universally available.² Critically ill adults can be transferred safely by fully equipped, specialised transfer teams; although these are common in Australia, North America, and some European countries, they remain the exception in the United Kingdom.

Table 1 Resources available at adult intensive care units for transfer of critically ill patients. Values are numbers (percentages)

Resources	1994 (n=198)	1986 (n=181*)
Staff:		
Escorted by senior house officer or registrar	175 (88)	
Escorted by anaesthetic staff	193 (97)	166 (92)
Non-anaesthetic medical escort	2 (1)	88 (49)
Escorted by on call staff	180 (91)	
Additional nurse escort	190 (96)	166 (92)
Equipment or monitoring:		
Dedicated ambulance	43 (22)	64 (35)
Dedicated trolley	41 (21)	38 (21)
Ventilator	188 (95)	129 (71)
Defibrillator	183 (92)	146 (81)
Electrocardiograph	195 (98)	165 (91)
Box of transfer drug†	195 (98)	164 (91)
Pulse oximetry	197 (99)	
Non-invasive blood pressure	187 (94)	
Invasive blood pressure	163 (82)	
Central venous or pulmonary artery pressure	126 (64)	
End tidal carbon dioxide	48 (24)	
Battery powered infusion devices	198 (100)	

*A 64% response rate.¹

†For intubation, anaesthesia, and cardiovascular resuscitation.

The persistent professional dissatisfaction with transfer arrangements probably reflects having to send inadequately trained medical escorts and the need for consultants to cover the emergency service in their absence. Targeted allocation of resources is required to reduce the number of transfers and provide a national system for safe secondary transportation of critically ill adults. A system of dedicated regional transport services would result in most patients being transferred by well equipped, experienced medical attendants and would spare staff on call at referring and receiving hospitals.

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Chlamydia pneumoniae antibodies and serum lipids in Finnish men: cross sectional study

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Chlamydia pneumoniae is an intracellular Gram negative bacterium that commonly causes respiratory infections. *C pneumoniae* infection has been associated with atherosclerosis in seroepidemiological studies, and the organism was recently found within atherosclerotic

lesions.¹ It is also known that acute infections interfere with lipid metabolism. Raised concentrations of triglycerides have been detected in Gram negative infections, and concentrations of high density lipoprotein cholesterol have been shown to decrease in both

Table 1 Serum triglyceride, total cholesterol, high density lipoprotein cholesterol concentrations (mmol/l) and ratio of high density lipoprotein cholesterol to total cholesterol in Finnish men according to *C pneumoniae* specific IgG antibody titres and smoking, adjusted for age

	Smokers† (n=506)			Non-smokers (n=542)			Total‡ (n=1053)		
	IgG <32	IgG 32-128	IgG ≥128	IgG <32	IgG 32-128	IgG ≥128	IgG <32	IgG 32-128	IgG ≥128
Triglyceride concentration (mmol/l; geometric mean)	1.18	1.17	1.18	1.00	1.12	1.14*	1.06	1.15	1.16
Total cholesterol concentration (mmol/l; mean)	6.51	6.46	6.47	6.25	6.32	6.33	6.36	6.38	6.41
HDL cholesterol concentration (mmol/l; mean)	1.27	1.25	1.24	1.36	1.23	1.24**	1.32	1.24	1.24**
Ratio of HDL to total cholesterol (geometric mean)	0.19	0.19	0.19	0.22	0.19	0.19**	0.21	0.19	0.19**

*P<0.05; **P<0.01 from analysis of covariance, adjusted for age.

†Includes occasional smokers and former smokers.

‡Data on smoking missing for 5 subjects.

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bacterial and viral infections.² We studied the effect of *C pneumoniae* infection on serum lipid concentrations.

Methods and results

The study population consisted of 1053 men who participated in a reindeer herders' health survey performed in Finland 1986-9.³ Blood samples were taken after 12 hours of fasting. Total cholesterol, high density lipoprotein cholesterol, and triglyceride concentrations were measured by routine enzymatic methods. Serum IgG and IgA antibodies specific for *C pneumoniae* antibodies were determined by the microimmunofluorescence method using *C pneumoniae* strain Kajaani 6 as an antigen. Analysis of covariance with age as a covariate was used to test concentrations of serum triglyceride, total cholesterol, and high density lipoprotein and ratios of high density lipoprotein cholesterol to total cholesterol in groups classified by smoking and antibodies specific to *C pneumoniae*.

The mean age of the study group was 47 (range 20 to 87) years; 32% (337) were current smokers. Both antibody prevalence and the age adjusted geometric mean antibody titres were significantly higher in smokers than in non-smokers; IgG antibodies were present (titre ≥32) in 83% (280/337) of smokers and in 77% (417/542) of non-smokers (P<0.01). Overall the geometric mean triglyceride concentration was 1.14 (range 0.32-9.07) mmol/l, the mean total cholesterol concentration 6.4 (3.1-11.2) mmol/l, and the mean high density lipoprotein cholesterol concentration 1.26 (0.56-3.11) mmol/l. The geometric mean ratio of high density lipoprotein cholesterol to total cholesterol was 0.19 (0.07-0.72). Triglyceride and total cholesterol concentrations were higher and high density lipoprotein cholesterol concentrations lower in smokers than in non-smokers. Table 1 shows age adjusted geometric means and ratios according to *C pneumoniae* specific IgG antibody titres and smoking. In non-smokers, triglyceride concentrations increased and high density lipoprotein cholesterol concentrations and high density lipoprotein cholesterol:total cholesterol ratios decreased significantly (P=0.017, 0.001, and 0.001, respectively) according to IgG antibody titres; values did not differ significantly in smokers. Overall, subjects positive for IgG had significantly higher triglyceride concentrations and lower high density lipoprotein

cholesterol concentration and the high density lipoprotein cholesterol:total cholesterol ratios (P=0.06, 0.003, and 0.007, respectively). Presence of IgA antibodies (titre ≥16) had only a minor association with lipid concentrations.

Comment

Changes in lipid metabolism are well recognised in the pathogenesis of atherosclerosis. High concentrations of triglycerides and low concentrations of high density lipoprotein cholesterol, as well as low ratios of high density lipoprotein cholesterol:total cholesterol, are known to be important risk factors for coronary heart disease.

The specific IgG antibodies in these men suggest that they have been infected by *C pneumoniae* or have a persistent *C pneumoniae* infection; the altered serum lipid values may thus reflect disturbances in lipid metabolism caused by infection. As a Gram negative bacterium, *C pneumoniae* contains lipopolysaccharide as a major constituent of its outer membrane. It can multiply in macrophages, smooth muscle, and endothelial cells and induce production of tumor necrosis factor and interleukin-1.¹ In macrophages and smooth muscle cells in atherosclerotic lesions *C pneumoniae* and its lipopolysaccharide may lead to continuous production of cytokines and thus to an altered serum lipid profile. Several other chronic bacterial infections such as *Helicobacter pylori* infection have recently been connected to atherosclerosis.⁴ If these infections contribute even partly to the altered serum lipid profile, eradication of the infection by antibiotic treatment would decrease the risk of atherosclerotic disease.

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